



THE CHEMISTRY OF DORYPHORA SASSAFRAS.

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Doryphora sassafras Endlicher, is the characteristic sassafras tree of New South Wales, as *Atherosperma moschatum* is of Victoria, and *Cinnamomum Oliveri* is of Queensland. Of these, the latter alone belongs to the Lauraceæ, the same Order as the true *Sassafras officinale* of North America; while *Doryphora* and *Atherosperma* are in the N.O. Monimiaceæ.

D. sassafras is indigenous to Eastern Australia, and is confined almost entirely to New South Wales. It begins in the south of Queensland, and extends southward almost to the Victorian border, while inland, it is limited by the Blue Mountains and the coastal ranges. It grows to an average height of 50 to 80 feet, but in some places has attained the height of 180 feet.

Aborigines, and also country people, make a tea from the bark, which they drink as a tonic. The light-yellow wood possesses the fragrance of the bark, and is not attacked by insects.

About a half hundredweight of bark was collected by Mr. W. H. Waters, near Fitzroy Falls, Moss Vale, in June, 1907, and was identified by Mr. R. T. Baker, Curator of the Technological Museum, from specimens of leaves and fruits. The fragrant odour of the bark in its fresh state was very strong, and during the drying, part of the volatile oil escaped, and the fragrance lessened, and finally became very faint. The air-dried material was laid aside at the time, until a convenient opportunity could be obtained to begin the investigation. During the three years' storage, a considerable portion of the most volatile constituents must have been lost, as the faint aromatic odour persisted throughout, and the air of the storeroom was constantly laden with the vapour.

PRELIMINARY ANALYSIS.

A small quantity of the powdered bark was first examined to ascertain the general characters of the constituents. It was passed through a 0.5 mm. sieve, and a weighed portion dried at 100°C., to constant weight. The fragrance during the heating was quickly replaced by a disagreeable odour, which persisted to the end. After weighing, the dried material was incinerated and the ash weighed. The following results were obtained:—

Air-dried bark.	
Loss at 100°C.—vol. oil and moisture ...	11.16%
Ash.....	3.48
Organic portion (by difference).....	85.36
	100.00

Action of various Solvents.—20 gms. of the same powdered sample were next extracted successively with various solvents.

Petroleum Spirit Extract.—This was evaporated to dryness at room temperature in a slow air-current, and the residue weighed. This residue possessed the fine fragrance of the volatile oil. When transferred to a desiccator, and the drying continued for a number of days, it continued to lose weight, and on the fourth day the fragrant odour had disappeared. The residue left was a thin pale yellow liquid. It was heated in the oven at 100°C., and attained a constant weight after 16 hours. This residue possessed a disagreeable, slightly pungent odour.

Weight of first dry residue.....	1.19%
„ after 4 days in desiccator	1.07
„ after heating to 100°C.....	0.40
	0.12
„ of fragrant essential oil.....	0.12
„ of other volatile oils	0.67

The residue, after heating, consisted of fixed oil, and a little resin. No alkaloids were found in the petroleum spirit extract.

Ether Extract.—When evaporated at room temperature, and dried in a desiccator, this amounted to 1.05%. Of this weight, 0.325 was volatilised by heating to 100°, and probably consisted of the same volatile oil as that obtained in the previous extract.

The fixed oil was dissolved out by petroleum spirit, 0.166%. Water removed in solution a part, which gave all the alkaloidal reactions, and there was left 0.234% of insoluble resins.

Volatile oil (vol. at 100°C.).....	0.325%
Fixed oil (sol. in petrol. spirit)....	0.166
Resins (insol. in water).	0.234
Alkaloid, etc. (diff. sol. in water)....	0.325
	1.050

Alcohol Extract.—From the residue, after petroleum spirit, and ether, alcohol extracts 3.2% of solid matter. From this, water removed a substance which gave a very persistent froth. Dilute ammonia dissolved a considerable part, and from this solution, when acidified, a brown deposit separated, mainly consisting of resins soluble in dilute alkali. The water-soluble portion gave a very pale green colour with iron alum, and gelatin solution gave only a small precipitate. The tannins were precipitated by lead acetate, and weighed, after deducting the lead oxide, 1.24%. The filtrate, after removing the lead, was tested with Fehling's solution, but no reducing substances were present either before or after hydrolysis with acid, showing the absence of glucosides. The alcoholic extract contains:—

Part insol. in water—resins.....	1.00%
PbA ppt.—tannins (chiefly)	1.24
Other substances.....	0.96
	3.20

Water Extract.—This contained 5% of material in solution. From it was separated, mucilage and dextrin in very small amounts. The amount of glucose, or reducing sugars obtained by Fehling's solution, and weighing the CuO, was 1%, and after hydrolysis by boiling with hydrochloric acid for 20 mins., 1.53% of glucose.

Total carbohydrate by hydrolysis....	1.53% expressed as glucose.
Reducing sugars	1.00 ,, ,,
Saccharose group.....	0.53 ,, ,,

The material left after the water extraction was then treated with 1% hydrochloric acid, in order to detect calcium oxalate. The extract was neutralised with ammonia, and precipitated with 2 vols. of alcohol. The flocculent brown deposit was dried and burnt, dissolved in acid, iron and other metals removed, the calcium precipitated as oxalate and titrated. The equivalent of calcium oxalate was 1.23%.

Results of the preliminary examination of air-dried bark : —

1.Extracted by petroleum spirit.	1.19%
2.Extracted by ether.	1.05
3.Extracted by absol. ethyl alcohol.	3.20
4.Extracted by distilled water.	5.00
	10.44

The approximate constituents found are :—

	(a)	(b)
Volatile essential oils.	1.117 per cent.	1.24 per cent.
Fixed oils	0.566 „	0.63 „
Resins.	1.234 „	1.37 „
Tannins (etc., pptd. by PbA.)	1.240 „	1.38 „
Reducing sugars (as glucose).	1.000 „	1.11 „
Saccharose sugars.	0.530 „	0.59 „
Calcium oxalate.	1.230 „	1.37 „
Alkaloid (approx.)	0.325 „	0.36 „

(a) Calculated on air-dry material; (b) on material dried at 100°C.

The prominent features brought to light by the above examination are (1) the existence of a fragrant essential oil, part of which was exceedingly volatile, and passed off into the air at ordinary room temperature. It was found impossible to volatilise the solvent from the ethereal or petroleum spirit solution, in the usual way by a current of air, without losing the greater part of the highly volatile constituents. (2) There was dissolved by alcohol a quantity of aromatic resins; and (3) a small amount of tannin. (4) The presence of an alkaloid was shown.

ANALYSIS OF THE INORGANIC PORTION.

The ash constituted 3.48% of the original bark.

3.93% of the bark dried at 100°.

The ash contained 18.33% soluble in water.

70.62% soluble in HCl.

11.05% insol. in HCl.

The entire ash was found to have the following composition, and is compared with the Victorian sassafras, *Atherosperma moschatum*, under the same conditions, from Zeyer's analysis(1).

	<i>Doryphora.</i>	<i>Atherosperma.</i>
Total ash.....	3.93	4.06
Na ₂ O.....	0.263	0.396
K ₂ O.....	0.102	0.164
MgO.....	0.069	0.177
CaO.....	1.450	1.845
Mn ₂ O ₃	0.013	0.019
Fe ₂ O ₃	0.070	0.004
Al ₂ O ₃	0.085	0.008
Cl.....	0.179	0.065
SO ₃	0.099	0.058
P ₂ O ₅	0.105	0.048
Insoluble.....	0.434	0.056
CO ₂	—	1.220

BULK EXTRACTION.

For the more complete examination of the constituents of the bark, a large quantity was next treated as in the following scheme:—

Extraction with alcohol.

Steam distillation of the extract, yielding

A. Volatile oil.

B. Aqueous distillate.

C. Insoluble resins.

D. Alkaloid in aqueous solution.

The air-dried bark, weighing 18 kilos., was passed through a powerful disintegrator, and the powder extracted twice with hot 95% methylated spirit. The extract was distilled under diminished pressure, and in the removal of the solvent much of the exceedingly volatile oil was unavoidably lost. The thick tarry liquid left in the still, and measuring about one and a half litres,

was transferred to a large flask, and distilled in a current of steam for many hours. A heavy yellow oil was carried over with the steam, and condensed. The oil was removed by a separating funnel, and the aqueous distillate was shaken up with petroleum spirit, which removed a further amount of oil existing as minute suspended globules. From the latter the solvent was removed, and the yellow oil added to the main portion. The residue in the retort consisted of resins, made insoluble by the loss of the essential oil in which they were originally dissolved, and the alkaloid partly in solution.

A. THE VOLATILE OIL.

The yield of oil from the steam distillation was 75.3 gms., equivalent to 0.42% of the bark. Since the figure given in the preliminary analysis, 1.117%, was obtained by difference, the following special assay was made to determine more exactly the amount present.

Special Assay.—200 gms. of the powdered bark were placed with water in a large distillation flask, and distilled in a current of steam until no more oil collected, using very efficient condensers with ice. The oil was separated, and that remaining suspended in the distillate was recovered by petroleum spirit; the whole weighed 2.06 gms. Equivalent to 1.03% of the bark, or 1.15% of the material dried at 100°.

A second supply of the bark freshly removed from the tree yielded 1.35% of volatile oil (calculated for the dried material).

Properties of the Essential Oil.—The oil was pale yellow in colour, and possessed the essential odour of the bark. It was neutral to litmus, phenolphthalein, and m. orange; heavier than water, having a density of 1.033 at 15/15°C. The optical rotation in a 1 dm. tube at 15°, $[\alpha]_D = +7.4^\circ$, and the refractive index at 15°, $[n]_D = 1.5258$.

Hydrochloric acid gas produced a bright purple colour, but no crystals formed showing the probable absence of cineol.

Bromine vapour gave first a red colour, which passed into blue, purple, and green; no crystals were formed.

The Essential Oil of the Fruits.—This was determined in the same way, and yielded 2% on the fresh fruits, or 4% on material dried at 100°. The crushed fruits emitted a strong odour of camphor.

COMPARISON OF ESSENTIAL OILS FROM THE BARK OF FOUR DIFFERENT
"SASSAFRAS" TREES.

	Yield.	Sp. gr.	Ref. index.	Rotn.	Safrol.
<i>Doryphora</i>	1.35 %	1.033 at 15°	1.5258	+7.4°	small amt.
<i>Atherosperma</i> (2)	1.20	1.042 at 14°	1.5274	+7.	small amt.
<i>Cinnamom. Oliv.</i> (3).	0.86	1.001 at 16°	...	+11.038	small amt.
<i>Sassafras offic.</i>	6 to 9	1.088	...	+3.26	80-90%

The numerical properties of the oils of *Doryphora* and *Atherosperma* are nearly the same, and it will probably be found that their constituents are the same. They differ entirely from the American sassafras oil of commerce, which has a much greater yield.

Fluckiger,(4) in 1888, stated that in both *Doryphora* and *Atherosperma*, the odour was strongly suggestive of safrol. Now in *Doryphora* oil, the stearoptene which crystallises out on freezing, and melts at about the same temperature as safrol, most probably represents the small amount of this constituent which is present. The recent investigation of the oil of *Atherosperma* by Miss Scott,(5) of Melbourne, shows that safrol is a constituent. The American oil, when cooled to 0°, becomes solid by the crystallisation of the very large amount of safrol contained in it(6).

Safrol is a constituent of the essential oils in typical members of the following Natural Orders—Monimiaceæ (*Doryphora*), Lauraceæ (*Sassafras*, *Cinnamomum*, *Beilschmiedia*), Magnoliaceæ (*Illicium*), Aristolochiaceæ (*Asarum*); and the chief supply for the world's market is made by the firm of Schimmel and Co., from *Cinnamomum camphora*.

B. THE AQUEOUS DISTILLATE.

The aqueous distillate, after the oil had been removed by ether, was found to have an acid reaction, and to contain no volatile alkaloid. Part of the solution was exactly neutralised with

baryta, evaporated, and heated to constant weight. The barium salt was then decomposed by sulphuric acid.

0.7065 gm. Ba salt gave 0.645 gm. $\text{BaSO}_4 = 53.73\%$ Ba; barium acetate requires 53.73% Ba.

The acid is, therefore, acetic acid alone. A part of the distillate was titrated with $\frac{N}{10}$ alkali, and from this, the amount of acetic acid in the whole distillate was found.

Total acetic acid 1.7 gms. = 0.01% of the bark.

= 2.27% of the volatile oil.

This acid is doubtless formed during the steam-distillation, by the partial hydrolysis of acetic esters existing in the original oil.

C. THE RESINS.

After the steam-distillation, the residue in the retort was removed while hot, and consisted of aqueous solution and a thick oily semi-solid mass. The latter, containing the resins and fixed oils, was washed repeatedly with hot acidulated water, and in this way the greater part of the alkaloid present was obtained in solution. The solid residue was dissolved in alcohol, and poured into a large volume of water. The sediment which settled was separated and dried.

Its weight was 350 gms., or 1.9% of the bark.

D. THE ALKALOID.

The aqueous solution containing the washings from the resins was concentrated to about 9 litres. This solution was treated with lead acetate, and then basic acetate, the precipitates being removed and washed. The filtrate, free from lead and hydrogen sulphide, was now concentrated to 4 litres, and the alkaloid separated by ammonium hydroxide. The voluminous alkaloidal precipitate being filtered off, the solution still contained alkaloid, which was then recovered by shaking with chloroform, and uniting it to the main precipitate.

Purification.—The crude alkaloid was now dissolved in dilute sulphuric acid, and precipitated with mercuric potassium iodide. From this, after careful washing, the alkaloid was recovered; it was then precipitated three successive times with ammonia, and

finally extracted with chloroform. This solution on evaporation to dryness left the alkaloid in an amorphous form, and of a yellowish-grey colour.

Attempts were made to obtain the alkaloid in the crystallised condition, but none of these were successful. Saturated solutions of the alkaloid, in water, methyl, ethyl, and amyl alcohols, ether, acetone, chloroform, and benzene, were allowed to evaporate spontaneously; amorphous residues were in all cases obtained. Salts of the alkaloid were next formed by neutralisation with sulphuric, nitric, picric and picrolonic acids; on spontaneous evaporation, not one of these was obtained in a crystallised form.

Properties of the Alkaloid.—The amorphous powder is highly electric; when brushing it out from one vessel to another, it either strongly adheres, or flies off and scatters.

The melting point lies between 115° and 117°C . It possesses a slightly bitter taste, and the reaction is faintly alkaline to litmus. It dissolves readily in alcohol, chloroform, and dilute acids; is very slightly soluble in ether, and water; and insoluble in petroleum spirit. The solutions are yellow to brown.

Concentrated sulphuric acid placed on a speck of the alkaloid on a white slab produces a pinkish-brown colour. No other colour reactions were observed. The alkaloid is precipitated from its salt solutions by ammonium hydroxide, sodium hydroxide, carbonate, and bicarbonate, picric acid and picrolonic acid, iodine, potassium mercuric iodide, tannic, phosphotungstic, and phosphomolybdic acids.

Titration of the Alkaloid.—Of the amorphous powder, 0.9614 gm. dissolved in hot water, required 28 cc. of $\frac{N}{10}$ sulphuric acid, = 0.1372 gm., to neutralise it to litmus.

0.1372 acid : 0.9614 alkaloid :: 49 : 343.

The equivalent weight of alkaloid is thus shown to be 343.

Assay of Bark for Alkaloid.—(a) 10 gms. of powdered bark were extracted in a soxhlet with alcohol. From the extract the solvent was distilled, the residue dissolved in dilute hydrochloric acid, water added, and filtered. From the filtrate the alkaloid

was precipitated by 9 cc. of Mayer's reagent.* This precipitate was decomposed with sodium sulphide, and the alkaloid obtained as hydrochloride. The solution was made alkaline with ammonia, and shaken out three times with chloroform. The chloroformic solution was evaporated in a weighed dish. The weight of alkaloid obtained was 0·0536 gm.

(b) 100 gms. of bark were extracted with hot alcohol as before. After removing the spirit, the residue was treated with water, and the resins filtered off. The small amount of tannin was separated by lead acetate, and the alkaloid obtained by precipitation with ammonia. The alkaloid which still remained in solution, was removed by agitating with chloroform, and added to the precipitate. The latter was then dissolved in alcohol, excess of standard acid added, and then titrated back to the neutral point with alkali, using sensitive litmus as indicated. Required 16·3 cc. $\frac{N}{10}$ acid.

(c) 10 gms. were treated as before, and the alkaloid obtained by agitating the aqueous solution with chloroform. The latter was evaporated, and the residue, which weighed 0·149 gm., was titrated. Required 1·55 cc. $\frac{N}{10}$ acid,

Results :—(b) 16·3 cc. $\frac{N}{10}$ sulphuric acid.

(c) 15·5 ,,

—————
mean 15·9 cc. = 0·0786 gm. sulphuric acid.

49 acid : 343 alkaloid :: 0·0786 : 0·55

therefore amount of alkaloid in bark... = 0·55 by titration.
 0·536 by weighing.

—————
calculated on air-dried bark, mean..... 0·54%

—————
and calculated on material dried at 100°C. 0·63%

Alkaloid in the Leaves and Fruit.—Samples of the bark, leaves and fruit were examined simultaneously by method (a). After distilling off the alcohol, and extracting with acidulated water, they were each titrated under the same conditions, with Mayer's reagent. The volumes required were

* Potassium mercuric iodide.

respectively—bark 100 cc., leaves 55 cc., fruit 20 cc.; so that the approximate amount of alkaloid in the leaves is 0.3%, and in the fruit 0.1% (calculated on the dried material).

Ultimate Analysis of the Alkaloid.—The following data were obtained by analysis of the amorphous powder, and must, therefore, be considered as provisional only.

Combustions.—

0.1808 gm. gave { 0.455 gm. CO₂, C = 68.64 per cent.
 { 0.102 gm. H₂O, H = 6.27 ,,
 0.1541 gm. gave { 0.3904 gm. CO₂, C = 69.09 ,,
 { 0.0886 gm. H₂O, H = 6.39 ,,

Nitrogen by Dumas' method.—

0.219 gm. gave 8.2 cc. N gas at 23.1°C. and 758 bar. N = 4.20%.
 0.193 gm. gave 7.2 cc. N gas at 21.2°C. and 766 bar. N = 4.27%.

Nitrogen by Kjeldahl's method using zinc dust, salicylic acid, potassium H sulphate, and sulphuric acid—

0.407 gm. required 12.6 cc. $\frac{N}{10}$ acid. N = 4.33%.

Results —

Found		Required for		
(i)	(ii)	C ₁₈ H ₂₁ NO ₄	C ₁₉ H ₂₁ NO ₄	C ₂₀ H ₂₁ NO ₄
C - 68.64	69.09	68.60	69.70	70.80
H - 6.27	6.39	6.66	6.42	6.20
N - 4.33	4.27	4.44	4.28	4.13
O - 20.76	20.25	20.30	19.60	18.90

C₁₈ is hydroxycodine, mol. wt. 315.

C₁₉ is tubocurarine, mol. wt. 327.

C₂₀ is papaverine and canadine... 339.

Physiological Action of the Alkaloid.—A definite weight of the amorphous alkaloid was converted into sulphate, and dissolved in normal saline. This solution was injected into the lymph sacs of frogs (*Hyla aurea* and *Limnodynastes*), and 1 mg. doses proved fatal. In 1 to 5 mins. after injection, the frogs became sluggish, their activity quickly decreased, so that they were soon unable to turn over, when laid on their backs. The voluntary muscles, first of the hind limbs, then of the fore limbs, were relaxed, and the reflexes disappeared entirely. In this general comatose condition, respiration

gradually became slower and weaker, till it ceased. In no cases were spasmodic reflexes or convulsions observed.

The effect on the heart was observed by exposing the heart of a pithed frog, and applying a 1 % solution of the alkaloid in normal saline. The beat became slower, and within 8-10 minutes stopped, with the ventricle in the systolic phase.

Muscle and nerve preparations were made from both *Hylas* and *Limnodynastes*. The muscle of one preparation and the nerve of another were laid in a watch-glass containing the solution of alkaloid, and the excitability tested. No alteration in the response was observed when nerve or muscle was stimulated by a faradic current.

COMPARISON WITH OTHER ALLIED PLANTS.

Of the twenty-two known genera of the Monimiaceæ, eight are represented in Australia; and of the latter, four—*Doryphora*, *Daphnandra*, *Palmeria*, and *Piptocalyx*, are found only in Australia.

About the year 1860, von Mueller sent a quantity of the Victorian sassafras, *Atherosperma moschatum*, to Professor Wittstein in Germany. It was handed over to the chemist Zeyer, who investigated its composition, and published his work in the *Jahresbericht* for 1861(7). The following comparison is made from an abstract in Wittstein's "Analyse von Pflanzen":—

The alkaloids of *Doryphora* and *Atherosperma* resemble one another in being precipitated by ammonia as bulky flocculent precipitates. When dry they are light, loose, highly electric powders, without odour, but possessing a bitter taste. Though almost white or pale gray in colour when first precipitated, they gradually become brown on exposure to light and air. They are nearly insoluble in water, and very faintly in ether highly soluble in alcohol, chloroform, and dilute acids. They are neutralised by acids giving varnish-like salts.

The two alkaloids differ in their melting points, *Atherospermine* m.p. 128°, while the *Doryphora* alkaloid m.p. is 115-117°.

Zeyer gave his alkaloid the formula $C_{30}H_{40}N_2O_5$ (old German $C_{30}H_{20}NO_5$), though he regarded it as doubtful at the time.

The provisional formula arrived at for the *Doryphora* alkaloid is $C_{18}H_{21}NO_4$.

In New Zealand there occurs another genus of the Monimiaceæ, *Laurelia Nova-Zelandeæ*, whose bark and leaves were found by Bancroft(8) to possess "an agreeable aromatic bitter taste." From this bark, Aston(9) isolated three alkaloids, one (laureline) having the formula $C_{19}H_{21}NO_3$, and m.p. 116° , was amorphous, but formed crystallised salts; another, was also an amorphous powder, from which no crystallised salts could be obtained. The physiological action of the chief alkaloid, as described by Professor Malcolm(10), shows first, a stage of increased excitability, quickly followed by complete loss of power and death. The second stage closely resembles that produced by the alkaloid of *Doryphora*

Piptocalyx Moorei(11), the "Bitter Vine" of New South Wales, owes its intensely bitter taste to a glucoside, which was examined by Umney(12) in London, and no alkaloids were detected.

The only other members of this Order, native to Australia, and whose constituents have been examined, are the three species of *Daphnandra*,—*repandula*, *micrantha*, and *aromatica*. Bancroft(13) has recorded the presence of bitter alkaloids in all parts of these plants, and found them to be powerful poisons. The physiological action on frogs resembled that of the *Laurelia*, producing convulsive movements, followed by paralysis.

Of the extra-Australian genera, *Monimia rotundifolia* was examined by Rochebrune(14) in 1897. He found in it a glucoside, a volatile oil and an alkaloid having properties almost identical with those of the constituents of the Chilean genus, *Peumus boldus*(15). The alkaloid of *Peumus* resembles that of *Doryphora* in many of its properties, both chemical and physiological, and it, too, could not be obtained in a crystallised form.

Therefore, it will be seen by these comparisons, that the alkaloid of *Doryphora* differs from that of the closely related genera in the absence of a first, or tetanic stage. But in all cases they are alike in their later stages. Many plants of the N.O. Lauraceæ, on the other hand, were shown by Greshoff (16) to contain laurotetanine, an alkaloid of the convulsive group.

RELATION TO OTHER ALKALOIDS.

(i) A consideration of the properties of the alkaloid from *Doryphora* brings out the following features. (a) The similarity of its properties to those of its nearest botanical ally—the atherospermine of Zeyer. (b) The molecular formula comes nearest to hydroxycodine, $C_{18}H_{21}NO_4$. This was obtained by Knorr, through the oxidation of codeine, and discovered by Dobbjæ and Lauder (17), last year, in the mother-liquor of the opium alkaloids, after all the other members had been eliminated. (c) The formula lies also very close to $C_{19}H_{21}NO_4$, given by Boehm (18) to tubocurare, which is a brown amorphous base, bitter and poisonous. (d) It also approaches that of Aston's laureline ($C_{19}H_{21}NO_3$), as well as (e) members of the hydrastine group of alkaloids. Of the latter group, canadine ($C_{20}H_{21}NO_4$), is a hydroberberine, an alkaloid found in the Berberidaceæ, Ranunculaceæ, and Menispermaceæ. It resembles berberine in the yellow colour of its powder, and solutions, as also that of its salts. (f) In the morphine group, the formula approaches papaverine ($C_{20}H_{21}NO_4$).

(ii) On the other hand, the properties of the alkaloid diverge from the characteristic properties of the above compounds, in the following way:—(a) The molecular formula, and melting point are far removed from Zeyer's figures for atherospermine. (b) It does not yield the characteristic colour reactions of codeine, and hydroxycodine melts at about 51° C. (c) It does not show the typical curare action on the receptive substance of the motor nerve endings in muscle, when administered to frogs. (d) Unlike laureline, it does not produce convulsions. (e) The physiological action excludes the hydrastine

group of alkaloids, for the same reason as in curare. (f) The physical properties are different from those of papaverine.

SUMMARY.

Doryphora sassafras is a small Monimiaceous tree, endemic in Eastern Australia. Its bark, leaves, and fruit contain an essential oil of characteristic sassafras odour. The oil has a density of 1.033, and distils between 60° and 230°C. The bark contains 1.35%, leaves 4.3%, and fruit 4% (% on material dried at 100°). The essential oil is compared with that from the Victorian sassafras, *Atherosperma moschatum*, the Queensland sassafras, *Cinnamomum Oliveri*, and the *Sassafras officinale* of N. America.

Other constituents of the bark are fixed oil 0.63%, aromatic resins 1.3%, tannins 1.3%, sugars 1.7%, calcium oxalate 1.37%, and an alkaloid 0.63% (% on bark dried at 100°).

The alkaloid is an amorphous grey powder. All attempts to obtain it, or its salts, in a crystalline form, were unsuccessful. The alkaloid is highly electric, m.p. 115-117°C., with a bitter taste and alkaline reaction; readily soluble in alcohol, chloroform, and dil. acids, very slightly in ether and water, insoluble in petroleum spirit. The solutions are yellow. Composition: C—68.64%, H—6.27%, N—4.33%, O—20.76%, corresponding closely to $C_{18}H_{21}NO_4$. The amount of alkaloid in the bark is 0.63%, in the leaves 0.3%, and in the fruit 0.1% (% on material dried at 100°).

The physiological action on the frog shows loss of power of movement, and of response to touch, paralysis and death. The min. lethal dose for *Hyla aurea*, a 13 gm. frog, is 1 mgm. No convulsions are produced, and the alkaloid has no action on nerve, receptive substance, or muscle.

The biochemical relationships of *Doryphora* are compared with other members of the same natural order. The alkaloid is compared with the active principles of allied plants, and also with alkaloids of approximately the same composition. After discussing the points of resemblance and difference in their properties, it is concluded that the alkaloid is a new one, and the name proposed for it is "DORYPHORINE."

Bentham and Hooker separate the Monimiaceæ and Lauraceæ with the monochlamydeous plants, but in Engler's classification we find the natural orders grouped closely together, which contain all the alkaloids mentioned above, except curare. From this comparison it is shown that the bark of *Doryphora sassafras* contains a new alkaloid, hitherto unrecorded, for which the name "Doryphorine" is proposed.

I wish, in conclusion, to express my indebtedness to Professor Anderson Stuart and Dr. Chapman, for affording every convenience to the carrying out of this investigation.

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POSTSCRIPT: *added July 3rd, 1912.*—The following observations, referring to the action of alkaloids on Australian frogs, were offered by Mr. E. C. Grey, B.Sc., Junior Demonstrator in Physiology, after the paper was read—With reference to Dr. Petrie's communication on the Chemistry of Doryphora, it was of importance to bear in mind that the behaviour of Australian frogs towards alkaloids had not yet been properly ascertained. Moreover the same alkaloid sometimes affected different frogs in different ways. He had observed the effect of Brucine on some Australian frogs, and had found that the strongly muscular *Limnodynastes* showed convulsions, whereas none were produced in any of the *Hylas* examined.